

## Iron Chelating Capacity of Gluconamides and Lactobionamides

### ABSTRACT

Various lactobionamides and gluconamides have been prepared by reaction of lactobiono- or glucono-lactones with amines. The amides were tested for their ability to chelate ferric ion in alkaline solution. In general, gluconamides were more effective chelators than corresponding lactobionamides.

### INTRODUCTION

Chelating and sequestering agents have had considerable application for many years. These materials usually form complexes or coordination compounds by reaction of their negatively charged donor groups with polyvalent metal ions such as iron, calcium, copper, and magnesium. The biological implications involving polynuclear iron complexes have been studied (5).

There are several classes of chelating agents including the EDTA (ethylenediamine tetraacetic acid) type and the aldonic ("sugar") acid group. Gluconic and citric acid are examples of the latter. The choice of chelating agent depends to a great extent upon the conditions under which it is applied. For example, the EDTA family is effective in acid, neutral, or alkaline media whereas the sugar acids function best at high pH (3). It is the carbohydrate family with which this note is concerned.

One of the most important sugar-based chelating agents is sodium gluconate, which has found extensive use as a chelating agent in the dairy, detergent, brewing, leather, and textile industries. Medicinally it is used as a carrier for calcium and iron in treatment of deficiencies of

these elements. Gluconates are prepared commercially by enzymatic transformation of D-glucose with glucose oxidase (EC 1.1.3.4,  $\beta$ -D-glucose:oxygen oxidoreductase) or by catalytic air oxidation of glucose. Higher members of the series such as sodium glucoheptonate can be prepared by addition of sodium cyanide to glucose followed by hydrolysis.

During recent years, scientists in this laboratory have been interested in new uses for lactose and its derivatives (4). Because of the similarity in structure to gluconic acid, lactobionic acid and its derivatives were expected to have analogous properties and applications. Lactobionic acid is prepared readily by oxidation of lactose microbially, electrolytically, or chemically. At present, no significant use of this compound or its derivatives has been demonstrated aside from limited application in pharmaceuticals as the calcium salt. We prepared various types of amides of lactobionic acid and gluconic acid to study and compare their iron chelating capacities.

### MATERIALS AND METHODS

Lactobiono-1,5-lactone was prepared from lactobionic acid (Aldrich Chemical Co.<sup>1</sup>) by repeated evaporation with a mixture of 2-methoxyethanol and toluene (8:1 by volume) as described by Isbell and coworkers (2). Solvent removal was effected by rotary evaporator. The lactone was recrystallized from 2-methoxyethanol and melted at 195 to 196 C. Glucono-1, 5-lactone (US Biochemical Corporation) was used as received.

Lactobionamides and gluconamides were formed conveniently as in (6) by reaction of glucono- or lactobiono-lactone with an amine such as propyl amine, ethylene diamine, or propargyl amine. For example, N-propyl lactobionamide was prepared by refluxing 5.1 g of the lactone (.015 mol) with 1.2 g (.02 mol) of propyl amine in 75 ml of anhydrous methanol.

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<sup>1</sup> Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

After 1 h the mixture was cooled to 0 C, filtered, washed with ethyl ether, dried, and recrystallized from ethanol. The amides were prepared in over 90% yield and are listed in Table 1. All amides gave negative ninhydrin tests except for monoamides formed from ethylene diamine. Structures of the amides were confirmed by their  $^{13}\text{C}$  NMR spectra.

#### Determination of Chelation Ability

Approximately 25 ml of a .10 M aqueous solution of the chelator were prepared. Then 1.0 ml of the solution was pipetted into a small flask to which a small amount (from 1.0 to 20.0 ml) of .10 M ferric chloride was added; 25.0 ml of 1.0 M sodium hydroxide then were added, and the mixture was shaken. If no ferric hydroxide formed, the solution remained clear, and larger amounts of ferric chloride could be chelated in subsequent experiments. Conversely, the appearance of ferric hydroxide indicated that a smaller volume of ferric chloride should be used in the next test. In this fashion a range of 1.0 to 20.0 ml of ferric chloride could be chelated depending upon the effectiveness of

the chelators tested. Results are in Table 1.

Other methods are available for measuring the chelating ability of sugars including one which uses radioactively-labeled ferric ion (1). However, our procedure is one which can be carried out readily with a minimum of equipment and gives results which are reproducible within a range of .50 ml.

#### RESULTS AND DISCUSSION

From Table 1, lactobionic acid and its amides were less effective as chelators for iron than were corresponding gluconic acid compounds. All gluconic acid derivatives gave values of 8.0 or above. The EDTA and lactosyl urea were ineffective under these conditions. The presence of an additional carboxyl group, as in saccharic acid or sodium mucate, afforded no benefits over gluconic acid in the amount of ferric chloride complexed. Amides formed by reaction of glucono- or lactobiono-lactone with 2-methyl-2-aminopropanol were slightly superior in chelating power to their parent acids. However, this was generally not the case with the other amides and diamides prepared and tested in

TABLE 1. Chelating capacity of sugar based amides and acids.

Chelator	Source	Vol. of .1 M FeCl <sub>3</sub> complexed —(ml)—
N-propyl lactobionamide	lactone + propyl amine	4.0
N-propyl gluconamide	lactone + propyl amine	11.0
N-propargyl lactobionamide	lactone + propargyl amine	5.0
N-propargyl gluconamide	lactone + propargyl amine	13.0
N,N'-ethylenebis-lactobionamide	2 mol lactone + 1 mol ethylene diamine	7.0
N,N'-ethylenebis-gluconamide	2 mol lactone + 1 mol ethylene diamine	14.0
N-aminoethyl-lactobionamide	1 mol lactone + 1 mol ethylene diamine	3.0
N-aminoethyl-gluconamide	1 mol lactone + 1 mol ethylene diamine	8.0
N-2-hydroxyethyl-1,1-dimethyl lactobionamide	lactone + 2-methyl-2-aminopropanol	7.0
N-2-hydroxyethyl-1,1-dimethyl gluconamide	lactone + 2-methyl-2-aminopropanol	15.0
Lactobionic acid	commercial	4.5
Gluconic acid	commercial	14.0
Saccharic acid	commercial	14.0
Sodium mucate	commercial	8.0
EDTA	commercial	1.0
Lactosyl urea	lactose + urea	1.0
2-Methyl-2-aminopropanol	commercial	1.0
2-Methyl-2-aminopropanol + lactobionic acid	commercial	4.5

this study. The 2-methyl-2-aminopropanol had no chelation power under the conditions of this test either alone or mixed with lactobionic acid.

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